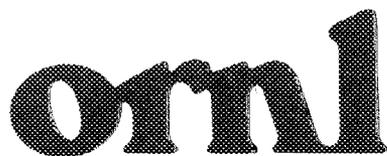




3 4456 0330584 9

ORNL/TM-11585



OAK RIDGE
NATIONAL
LABORATORY



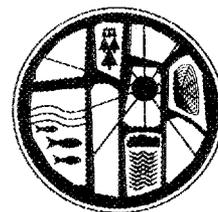
**Literature Review and
Preliminary Assessment of
Biological Transformations and
Biotreatment Technology for
Petroleum Hydrocarbons and
Chlorinated Solvents**

N. E. Korte

Environmental Sciences Division
Publication No. 3513

OAK RIDGE NATIONAL LABORATORY
CENTRAL RESEARCH LIBRARY
CIRCULATION SECTION
4370A BLDG 173

LIBRARY LOAN COPY
DO NOT TRANSFER TO ANOTHER PERSON
If you wish someone else to see this
report, send to them with report and
the library will arrange a loan.



MANAGED BY
MARTIN MARIETTA ENERGY SYSTEMS, INC.
FOR THE UNITED STATES
DEPARTMENT OF ENERGY

ORNL/TM-11585

ENVIRONMENTAL SCIENCES DIVISION

LITERATURE REVIEW AND PRELIMINARY ASSESSMENT OF BIOLOGICAL
TRANSFORMATIONS AND BIOTREATMENT TECHNOLOGY FOR PETROLEUM
HYDROCARBONS AND CHLORINATED SOLVENTS

N. E. Korte

Environmental Sciences Division

Publication No. 3513

Date Published: December 1990

Prepared by the
OAK RIDGE NATIONAL LABORATORY
Grand Junction Office
P.O. Box 2567
Grand Junction, Colorado 81503
managed by
MARTIN MARIETTA ENERGY SYSTEMS, INC.
for the
U.S. DEPARTMENT OF ENERGY
Under contract DE-AC05-84OR21400

MARTIN MARIETTA ENERGY SYSTEMS LIBRARIES



3 4456 0330584 9

CONTENTS

FIGURES	v
ABSTRACT	vii
1. INTRODUCTION	1
2. BASIC CONCEPTS OF BIOREMEDIATION	2
2.1 BACKGROUND	2
2.2 CONDITIONS FOR BIOCHEMICAL DEGRADATION.....	2
2.3 SUBSURFACE MICROBIAL ENVIRONMENT	4
3. ABIOTIC PROCESSES.....	6
4. BIOLOGICAL DEGRADATION PROCESSES	9
4.1 ANAEROBIC PROCESSES	9
4.1.1 Chlorinated Solvents	9
4.1.2 Petroleum Hydrocarbons	13
4.2 AEROBIC PROCESSES	14
4.2.1 Chlorinated Solvents	14
4.2.2 Petroleum Hydrocarbons	16
4.3 SEQUENTIAL ANAEROBIC/AEROBIC DEGRADATION	18
4.4 UNRESOLVED ISSUES	18
4.4.1 Microbial Activity	19
4.4.2 Compound-Specific Effects	20
4.4.3 Solution Effects	21
4.4.4 Interface Effects and Migration of Microbes	22
4.4.5 Contaminant Availability	22
4.4.6 Acclimation Period	22
4.4.7 Kinetics	23
5. BIOREMEDIATION CONCEPTS	24
5.1 GENERAL	24
5.2 UNSATURATED SYSTEMS	24

5.3	SATURATED SYSTEMS	25
5.4	MODELING	28
6.	COMMERCIAL PROCESSES	32
6.1	COMPANY A	32
6.2	COMPANY B	33
6.3	COMPANY C	34
6.4	COMPANY D	34
6.5	COMPANY E	35
6.6	COMPANY F	35
6.7	COMPANY G	36
6.8	COMPANY H	36
6.9	COMPANY I	37
6.10	SUMMARY OF INFORMATION FROM COMMERCIAL VENDORS	37
6.11	TREATMENT COSTS	38
7.	CONCLUSIONS	39
	REFERENCES	40
	SELECTED BIBLIOGRAPHY	57

FIGURES

<u>Figure</u>		<u>Page</u>
1	Three general conditions for biochemical degradation of organic compounds (Richards and Shieh 1986).....	3
2	Abiotic transformation of trichloroethane and tetrachloroethane (Maskarinec et al. 1989).....	8
3	Transformations of trichloroethene.....	11
4	Plan view for idealized in-situ biotreatment system (Cunningham, Characklis and Bouwer 1988a).....	26
5	Illustration of the numerous variables involved with modeling biodegradation (Modified from Cunningham, Characklis and Bouwer 1988a).....	29

ABSTRACT

Chlorinated solvents and petroleum hydrocarbons may undergo a number of natural degradation processes when applied to soil or groundwater. Indeed, the existence of these reactions has led to extensive research and the development of biodegradation as a remedial action technique. Unfortunately, the scientific literature demonstrates that there is considerable controversy concerning many aspects of the field. For example, different investigators are often unable to agree on relative rates of biodegradation or even whether certain compounds are biodegradable.

This report examines the recent scientific literature, describes the biodegradation reactions that are known to occur, and discusses some of the controversies. The potential value of biodegradation for remedial action of soils and groundwater is also presented both from a review of the literature and from interviews with remedial action contractors.

1. INTRODUCTION

This report provides an overview of chemical and biological degradation processes undergone by chlorinated solvents and petroleum hydrocarbons in soils and groundwater. The intended audience for the report is geoscientists, engineers, and project managers responsible for sites contaminated with chlorinated solvents and petroleum hydrocarbons. The review is derived from an thorough examination of recent literature. The information is presented within the context of considering biodegradation as a remedial technique for sites contaminated with hazardous waste. The final portion of the report, therefore, presents a discussion of remedial action experience as determined from interviews with representatives of firms that are actively marketing bioremediation.

The need for the report stems from the explosive growth undergone by bioreclamation in the past 5 years. Research projects have been performed by many industrial, governmental, and university entities. As might be expected for such a rapidly growing field, the results of the various research groups have provoked considerable controversy. Indeed, the technical literature is replete with contradictory findings.

The report compares and contrasts some of the contradictions but also presents established facts so that data from site investigations can be correctly interpreted and satisfactory remedial actions can be implemented.

2. BASIC CONCEPTS OF BIOREMEDIATION

2.1 BACKGROUND

The concept of using microbial activity to remove contamination is not new. Indeed, biological processes have been used in most publicly owned wastewater treatment works (Portier and Ahmed 1989). Only in the last decade, however, has there been an extensive effort to apply microbial processes to waste-contaminated soil and groundwater as opposed to municipal wastes. Proliferating investigations into subsurface microbial activity led to the discovery of new organisms and proof that microbial activity can be found in the subsurface to depths of several hundred feet (Streile et al. 1987).

2.2 CONDITIONS FOR BIOCHEMICAL DEGRADATION

The biochemical breakdown of organic compounds takes place under three general conditions (Fig. 1) (Richards and Shieh 1986): aerobic respiration, anaerobic respiration, and fermentation. Aerobic respiration occurs in the presence of oxygen, where compounds degrade through the action of oxygenase enzymes. Oxygen is also incorporated into the final products. Anaerobic respiration occurs in the absence of oxygen, with microbes using inorganic electron acceptors to metabolize substrates. In anaerobic systems, organic carbon is reduced to methane, nitrate to nitrogen, and sulfate to sulfide. Fermentation also occurs under anaerobic conditions but does not use an external electron acceptor. Fermentation only slightly reduces compounds, requiring aerobic processes to complete the degradation.

An important mechanism which does not conveniently fit into one of

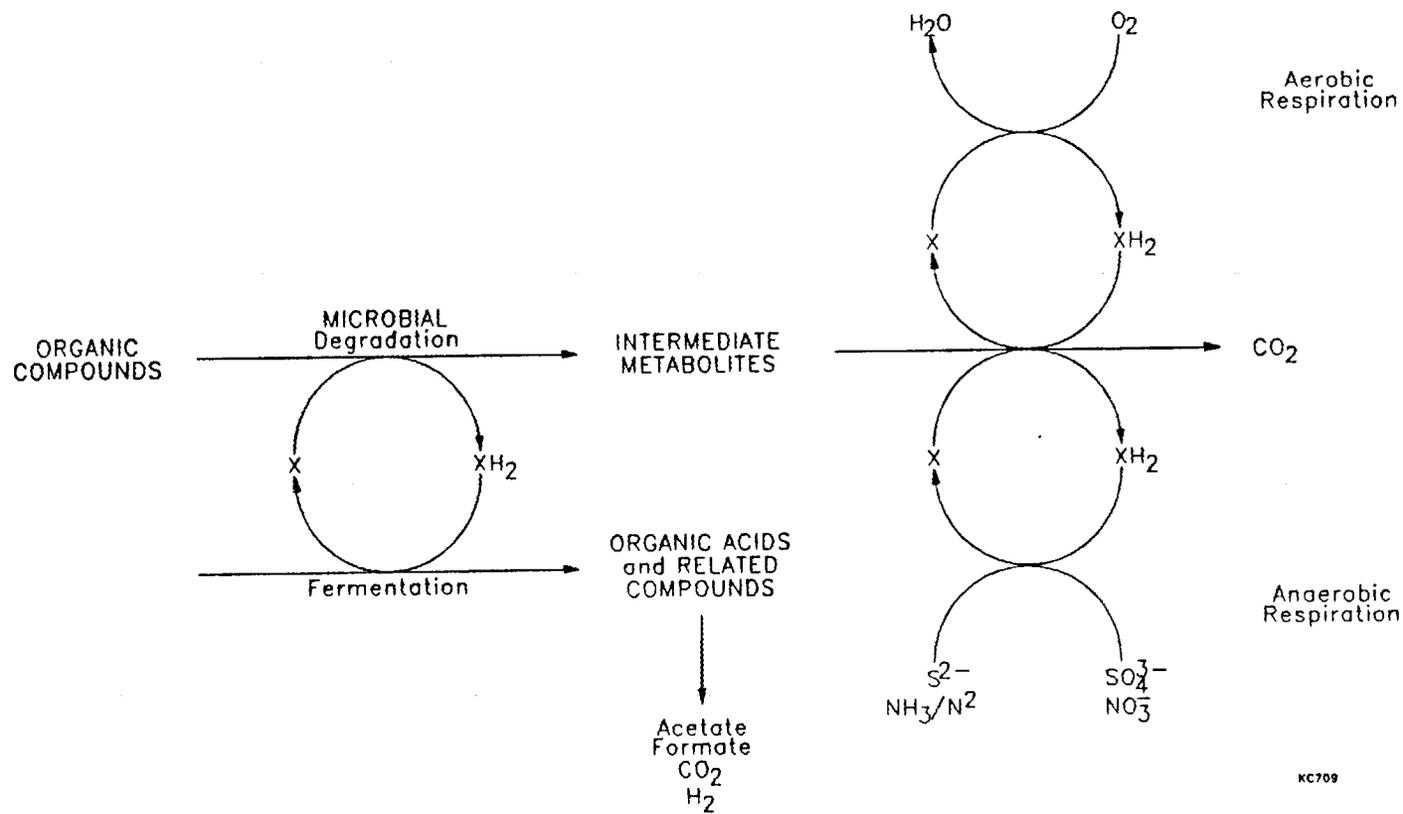


Fig. 1. Three general conditions for biochemical degradation of organic compounds (Richards and Shieh 1986).

the three general categories is cometabolism. A cometabolic process is one in which the microorganisms are utilizing another substrate rather than the compound of interest. Indeed, the latter compound may be toxic or simply not capable of supporting microbial growth. Nevertheless, the microorganisms may be growing so actively that they inadvertently consume and transform compounds that would not otherwise undergo biodegradation.

Biotransformation can occur slowly or rapidly, depending on site-specific conditions that are generally difficult to sort out on an a priori basis (Jafvert and Wolfe 1987). Indeed, at some sites, pesticides and herbicides biodegrade so quickly that the compounds are ineffective (Reed et al. 1989).

2.3 SUBSURFACE MICROBIAL ENVIRONMENT

Aerobic and anaerobic bacteria, fungi, actinomycetes, algae, and cyanophytes (blue-green algae) are all capable of degrading many classes of organic chemicals. Microbes used in remedial action might include natural microbial populations, adapted microbial cultures, and, potentially, bioengineered microbial strains (Amdurer et al. 1985). Subsurface bacteria are small, generally ranging in size from 0.5 to 1 μm (Kindred and Celia 1989).

In aerobic soils, heterotrophic bacteria (which use organic compounds as an energy and carbon source and use molecular oxygen, nitrate, or sulfate as an electron acceptor) usually dominate the microbial population. Methanotrophs, a special group of aerobic bacteria, use primarily methane or methanol as energy and carbon sources. Methanotrophic bacteria are of particular interest because of their ability to cometabolize (not use for growth) some organic contaminants commonly found in groundwater.

Eventually, groundwater not replenished with oxygen-rich surface

water will, through aerobic bacterial activity, become anaerobic. At very low or zero oxygen, some aerobic bacteria switch to nitrate or other forms of oxidized nitrogen. When both oxygen and oxidized nitrogen are depleted, the dominant microbes are anaerobic fermentative bacteria living in symbiosis with methanogens or sulfate-reducing bacteria (Kindred and Celia 1989). If the dominant metabolic groups inside an anaerobic contaminant plume are methanogens (methane-producing) anaerobes, methanotrophs may be quite active in aerobic soils surrounding the plume.

3. ABIOTIC PROCESSES

Several abiotic processes remove organic contaminants from soil and water; volatilization is the most important. Indeed, in some circumstances, removal of contaminants through abiotic processes is of primary importance in assessing the degradation characteristics of a particular water/soil system.

Unlike chemical transformations, microorganisms typically require time to acclimate to a contaminant before active growth can occur. The absence of an acclimation period typically indicates an abiotic reaction. Abiotic reactions are most common for compounds containing larger halogens (bromine or iodine) or for compounds with extensive halogenated substitution. For example, Bouwer et al. (1981) show that brominated trihalomethanes degrade chemically, and Jafvert and Wolfe (1987) reported that hexachloroethane reduces abiotically. Similarly, Curtis and Reinhard (1989) reported hexachloroethane reduced to tetrachlorethene. Curtis and Reinhard (1989) reported that humic and fulvic acids may serve as the electron acceptors for these reactions. Thus, the reactions may be controlled by the amount and nature of natural organic matter.

Trichloroethene (TCE) may lose a chlorine to form one of the dichloroethenes (DCEs) (1,1-, cis-1,2-, or trans-1,2-DCE) by a process called reductive dehalogenation. Typically, this is a biological reaction, but evidence exists that in aerobic systems the process can occur abiotically (Curtis and Reinhard 1989). Abiotic reductive dehalogenation of TCE, as mentioned, is apparently never an important reaction.

In contrast, Schwarzenbach et al. (1985) studied highly anaerobic systems and found that, in the presence of hydrogen sulfide, certain volatile halogenated alkanes in groundwater undergo nucleophilic substitution leading to the formation of persistent and hazardous

dialkyl sulfides. Such reactions were common for bromides and iodides. Chlorinated compounds can undergo these reactions, but they are not as common in aromatic or vinylic compounds.

Of greatest interest are possible abiotic transformations by the common degreasing solvent 1,1,1-trichloroethane (1,1,1-TCA). This substance degrades abiotically to 1,1-DCE at neutral pH and 20° C (Vogel and McCarty 1985). Cline et al. (1986), using field reports from sites in Florida (where water temperatures are relatively high), determined that high temperatures promote the transformation. Furthermore, pH had no effect on the transformation, nor were any specific redox conditions required, indicating the classic dehydrohalogenation reaction discussed in organic chemistry textbooks (Morrison and Boyd 1987). In the laboratory, complete abiotic conversion of 1,1,1-TCA to 1,1-DCE occurs in a matter of days (Fig. 2, Maskarinec et al. 1989), a reaction known not only for 1,1,1-TCA and hexachloroethane but also for 1,1,2,2-tetrachloroethane, which can dehydrohalogenate to form 1,1,2-trichloroethene (Cooper et al. 1987).

Dehydrohalogenation reactions typically will not dominate a particular site, either because biological reactions are much faster or because the temperature is too low for the reaction to occur. However, such reactions may dominate at high-temperature sites, particularly if organic matter is largely absent, thereby providing no substrate to support microbial growth.

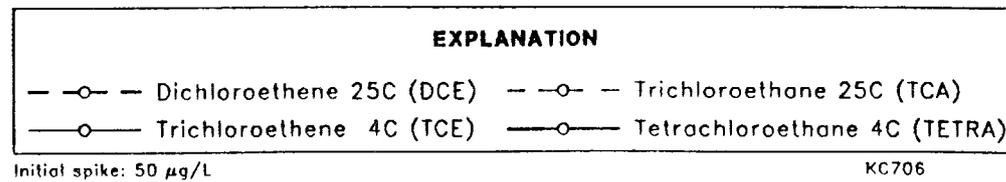
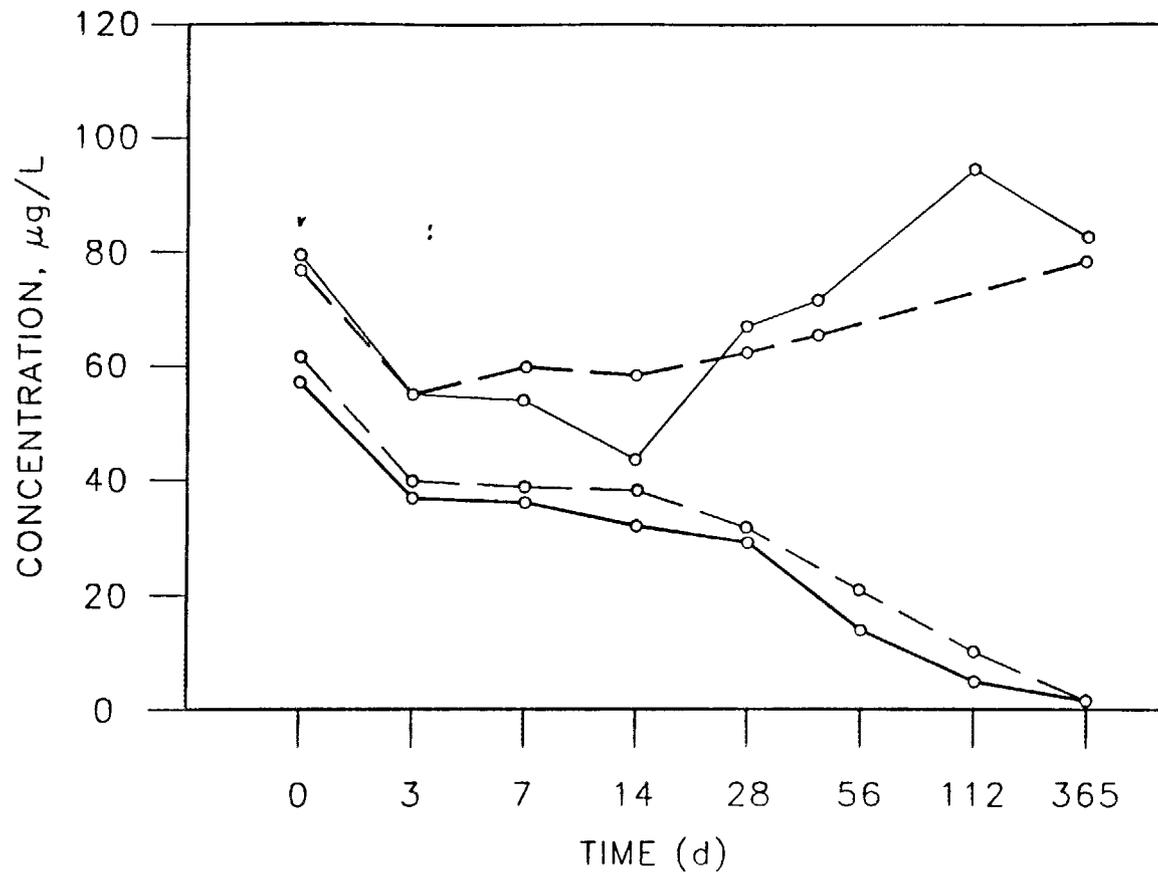


Fig. 2. Abiotic transformations of trichloroethane and tetrachloroethane.

4. BIOLOGICAL DEGRADATION PROCESSES

4.1 ANAEROBIC PROCESSES

More organic compounds have been shown to undergo anaerobic rather than aerobic biodegradation in the environment. Even complex pesticides such as toxaphene degrade anaerobically (Mirsatori et al. 1987). Nitrilotriacetic acid (NTA) can degrade either aerobically or anaerobically, but the process is much faster anaerobically (Ward 1986). Phenolic compounds (Suflita and Miller 1985) and ethylene dibromide (Pignatello 1987) also may degrade aerobically or anaerobically.

Some compounds degrade only under narrow redox conditions. For example, chloranilines biologically dehalogenate under methanogenic conditions but not under sulfate-reducing conditions (Kuhn and Suflita 1989). Dichlorobenzenes (DCBs) dehalogenate readily, but monosubstituted aromatics do not reductively dehalogenate. This may be the reason that chlorobenzene, along with xylene (probably *o*-xylene), is frequently the principal contaminant remaining at old fuel spills.

4.1.1 Chlorinated Solvents

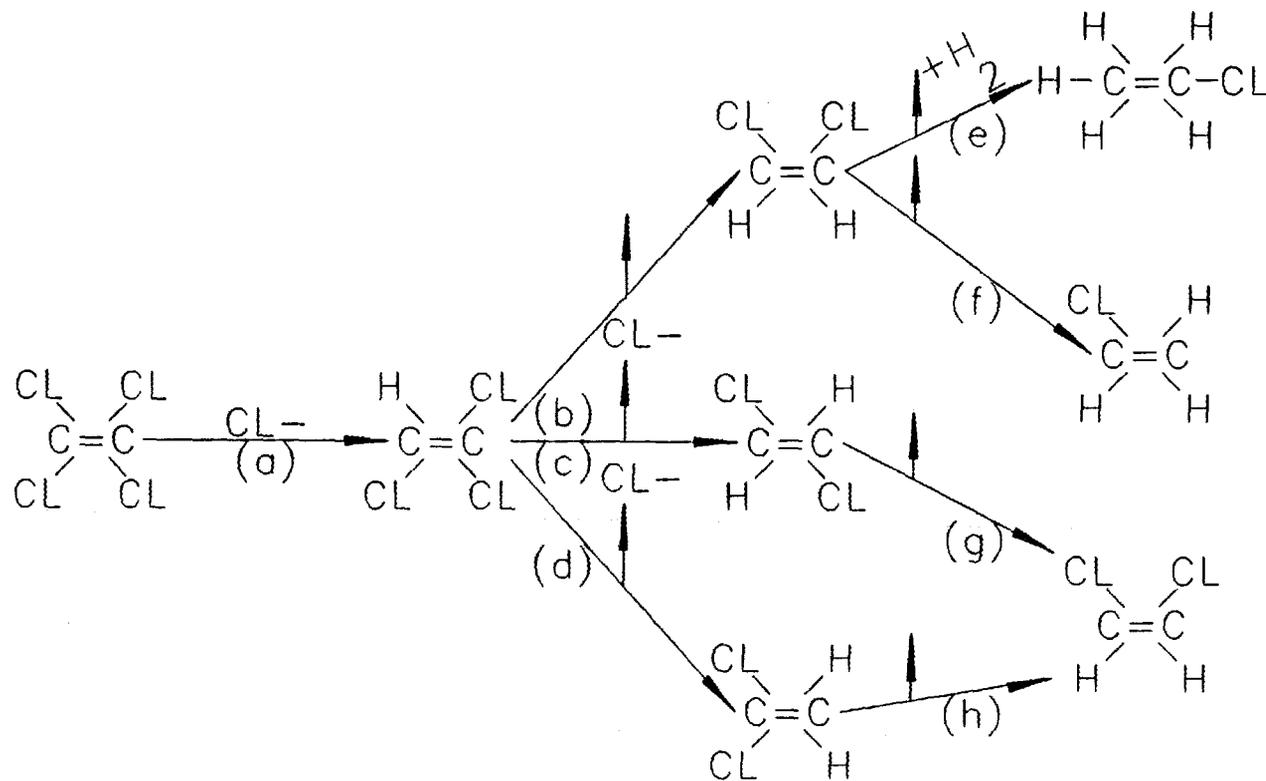
Anaerobic degradation of chlorinated solvents was discovered after aquifers were found to be contaminated with compounds that had not been used at the sites. The anaerobic degradation of TCE has now been reported by many investigators. Reportedly, under sulfate-reducing methanogenic conditions, all dichlorobenzenes, TCE, and tetrachloroethene (PCE) undergo reductive dechlorination (Bosma et al. 1989).

Vogel and McCarty (1985) showed that PCE and TCE degrade to vinyl chloride (VC) in 10 d under methanogenic conditions. Similarly, Jaffe and Baek (1989) show that TCE first degrades to 1,1-DCE, then to VC, and

finally to chloroethane. The increase in the amount of methane in the headspace of vials containing the microcosm as the concentration of degradation products built up in solution indicates the process was methanogenic. These authors also reported that ratios of DCE to VC to chloroethane differ significantly, depending on the methanogenic activity of the aquifer. If methanogenic activity is very high, then VC dominates. Under typical conditions, however, there will be more DCE than VC, which will be much more prevalent than chloroethane. In fact, the latter is detected only occasionally because it reacts rapidly once formed. This mechanism indicates that anaerobic processes may eventually clean an aquifer. Mechanistic studies show clearly that the optimum configuration for dechlorination is a dichloromethyl group (Van Dyke 1977). Thus, the final step, degradation of VC, is so slow that aerobic processes are usually needed to complete the biodegradation.

Several authors have used organic substrates to study anaerobic degradation. An early column study used a 2-d retention time and indicated that 90% of the chlorinated solvents were utilized with acetate as the primary substrate (Bouwer and McCarty 1983). However, Barrio-Lage et al. (1987a,b) reported that soil organic matter has no effect on the anaerobic transformation of TCE. Finally, in a study excluding chlorinated solvents, dibutylphthalate, which degraded either aerobically or anaerobically, was stimulated by the addition of organic matter (Inman et al. 1984).

There has been considerable controversy over which DCE isomer is formed from TCE (see Fig. 3). For example, Kloefer et al. (1985) found only 1,2-DCE (no 1,1-DCE). Barrio-Lage et al. (1987a) found only cis-1,2-DCE. Parsons et al. (1984) reported the formation of cis- and trans-1,2-DCE and 1,1-DCE from TCE, noting that the cis compound was formed more often than 1,1-DCE, which in turn dominated over the trans compound. Cline and Viste (1985) indicated that PCE goes to TCE, which degrades primarily to 1,1-DCE with some cis- and trans-1,2-DCE. Jaffe and Baek (1989) attempted to resolve the controversy by reporting that



- (a) Formation of trichloroethene from tetrachloroethene
- (b) Formation of cis-1,2,dichloroethene from trichloroethene
- (c) Formation of trans-1,-2,dichloroethene from trichloroethene
- (d) Formation of 1,1,dichloroethene from trichloroethene
- (e) Formation of chloroethane from cis-1,2,dichloroethene
- (f) Formation of chloroethene (vinyl chloride) from cis-1,2,dichloroethene
- (g) Formation of chloroethene (vinyl chloride) from trans-1,2,dichloroethene
- (h) Formation of chloroethene (vinyl chloride) from 1,1,dichloroethene

Fig. 3. Transformations of trichloroethene.

KC708

1,2-DCE is formed at higher pH and 1,1-DCE at lower pH. This pH effect, however, was not quantified.

Other anomalies besides the confusion over which DCE isomer is formed have been reported. For example, Barrio-Lage et al. (1986) reported that chloroethane forms only from cis-1,2-DCE--a finding not discussed by other investigators.

Conditions initiating degradation reactions are also not well understood. Parsons et al. (1985) suggested an Eh threshold after which degradation of PCE and TCE to VC occurs rapidly. Furthermore, the amount of contaminant needed for degradation to occur is also unknown. Very low contaminant concentrations might not stimulate the microbes, and degradation will not occur.

The only irrefutable conclusion based on these data is that chlorinated ethenes transform under anaerobic conditions, apparently with several simultaneous removal reactions (Fig. 3, Barrio-Lage et al. 1986). Such contrasting results demonstrate why a simple "degradation analysis," employing ratios of daughter to parent products, is unlikely to provide any useful information (Looney and Marine 1986).

Finally, there remains much controversy over both the biodegradability and the reaction mechanisms for many of the compounds. For example, Bower et al. (1981) stated that dichlorobenzene and PCE are not degradable under denitrifying conditions, but Bae et al. (1989) state that they are degraded. Various authors report that the biodegradation of 1,1,1-TCA is slow (Cline and Viste 1984), relatively rapid (Arvin 1989), or nonexistent (Wilson et al. 1989). Uncertainties with respect to reaction mechanisms are illustrated by a recent report which showed that a single organism was capable of dehalogenating 1,1,2-TCA by both oxidative and reductive pathways at the same time (Castro and Belser 1990). Bae et al. (1989) believe that the reason for much of the controversy concerning which reactions occur and which do not is that researchers have used different flow rates or reaction times in the laboratory studies. Other factors which may explain the variable

results are the use of different cultures and different concentrations.

4.1.2 Petroleum Hydrocarbons

Aromatic compounds are generally the chief interest in a fuel spill because such compounds make up 93% of the soluble fraction while accounting for less than 50% of the weight. In a comprehensive review of fuels biodegradation, Atlas (1981) stated that "current evidence supports the view that anaerobic degradation by microorganisms at best proceeds at negligible rates in nature." It is now known, however, that aromatics degrade both aerobically and anaerobically (Berry et al. 1987).

Anaerobic degradation of toluene and xylene may occur with sulfate as the electron acceptor. Toluene degrades first, then benzene, then *p*-xylene and *m*-xylene, which degrade much faster than *o*-xylene (Goldsmith and Balderson 1988, Alvarez et al. 1989). These results contrast with those from an extensively studied site in Traverse City, Michigan, where Reinhard et al. (1984) reported that alkylbenzenes such as xylene degrade preferentially over other fuel hydrocarbons.

Denitrifying conditions have been studied in the laboratory as a means of stimulating microorganisms to degrade aromatics (Hutchins and Wilson 1989). Under denitrifying conditions xylenes can apparently be used as a sole-carbon source (Kuhn et al. 1985).

Anaerobic conditions were thought to prevent the degradation of halogenated aromatics, but it has been shown that reductive dechlorination will occur (Schraa et al. 1989). Indeed, Boyd (1987) found that aromatic dechlorination does not occur unless there is anaerobic biological activity. As an example, pentachlorophenol lasts for months in an oxic soil but degrades rapidly under anaerobic conditions. The reactions are difficult to study, however, because of an apparent threshold concentration that must be reached before any biodegradation occurs. This threshold is site and compound specific,

depending on the other compounds present (Wilson et al. 1989), the organism, and the soil and water conditions.

4.2 AEROBIC PROCESSES

4.2.1 Chlorinated Solvents

The study of aerobic degradation of chlorinated solvents has undergone tremendous change in the past decade. Initial studies reported that aerobic degradation of compounds such as TCE was not possible (Bouwer et al. 1981, Wilson and McNabb 1983, Bouwer and McCarty 1983, Kuhn et al. 1985, Richards and Shieh 1986). In 1985, however, Wilson and Wilson (1985) reported the degradation of TCE in unsaturated soil by methanotrophic microorganisms. Likewise, Little (1987) and Little et al. (1988) reported TCE biodegradation by aerobic pure cultures of methanotrophs. The mechanism for the aerobic biodegradation of TCE involves oxidation of the TCE (Henry and Grbic-Galic 1989), which results in the formation of an epoxide intermediate (Arvin 1989). Some controversy remains, however, over whether the major role of the methane-oxidizing bacteria in this degradation involves stimulation of the microbial community or the direct consumption of TCE (Phelps et al. 1988).

Several other researchers (Fogel et al. 1986, Moore et al. 1989, Barrio-Lage et al. 1988) have also reported success with aerobic degradation, especially when adding methane and oxygen to stimulate the microorganisms. Research shows that a wide variety of chlorinated solvents degrade aerobically, including methylene chloride, chloroform, VC, 1,2-dichloroethane, 1,1,1-TCA, cis- and trans-1,2-DCE, TCE, and ethylene dibromide (EDB). All can be degraded when the concentration is as high as 5 mg/L (Moore et al. 1989). Some of these compounds undergo reductive dehalogenation anaerobically as well, but VC--a known

carcinogen--forms in some cases. Thus, a major advantage of aerobic degradation is that VC is not formed (Garland et al. 1989, Mayer and Grbic-Galic 1989).

Current research focuses on methods for maintaining aerobic bioreactors for use in remedial action. Bouwer et al. (1989) have conducted a 3-year bioreactor experiment, which shows the need for an adequate supply of electron acceptors. Aerobic biodegradation ceased if the nitrate or sulfate became depleted. Sulfate, however, can also inhibit aerobic biodegradation (Barrio-Lage et al. 1988). Bouwer et al. (1989) reported, as have Mayer and Grbic-Galic (1989), that low oxygen solubility in water greatly limits aerobic biotransformation. Because of the difficulty of maintaining oxygen in the system, hydrogen peroxide was added. Unfortunately, hydrogen peroxide's use appears limited because it reacts with natural organic matter, iron, and manganese.

As with anaerobic systems, different research groups report varying results on relative rates of biodegradation. For example, 1,1,1-TCA is usually considered slowly biodegradable (Bae et al. 1989), but some have reported that it degrades as fast as TCE (Arvin 1989). Arvin further reported that TCE, 1,1,1-TCA, and cis-1,2-DCE degrade at about the same rate--all much slower than trans-1,2-DCE. Indeed, many believe that the "slowness" of these rates is an obstacle to effective practical use of aerobic biodegradation. In contrast, Hanson et al. (1989) reported the rate of aerobic biodegradation to be VC > cis-1,2-DCE > TCE > trans-1,2-DCE, with PCE and 1,1-DCE not being degradable.

A lower methanotrophic population may explain the difference between the nearly complete TCE degradation reported by researchers using methanotrophic cultures grown with a supply of gaseous methane and studies conducted without any gas phase. With the low biomass supported on dissolved methane, the amount of monooxygenase enzyme present might simply be insufficient for complete and rapid TCE degradation. For example, in a preliminary column experiment, the crude estimates possible without destructive sampling yielded a population of

approximately one million organisms per milliliter, several orders of magnitude less than the populations measured by researchers reporting complete TCE degradation (Mayer and Grbic-Galic 1989). Indeed, these authors suggest adding methanol or formate when the methane is depleted to keep the population of methanotrophs high. Others have reported that the addition of substrates seems to have no effect, as shown in one experiment using methanol and acetate additions (Bosma et al. 1989).

Perhaps the results differ because the stimulation of organisms with additions is very difficult to maintain in a uniform fashion (Henry and Grbic-Galic 1989), even though the oxygen content may vary from 1 to 6 mg/L without any harmful effects (Arvin 1989). Adding methane up to about 5%, of course, is also necessary to stimulate the process; however, if more is added, biodegradation ceases (Hanson et al. 1989).

Temperature effects may also cause some of the reported variability. For example, a temperature decrease reportedly slows degradation, but after time the microorganisms may compensate and begin growing as rapidly as before (Bosma et al. 1989).

4.2.2 Petroleum Hydrocarbons

The study of aerobic degradation of petroleum hydrocarbons is extensive because these substances degrade easily and petroleum spills are prevalent. The complexity of petroleum hydrocarbon mixtures, however, makes it necessary to address their various components.

N-alkanes are generally considered the most readily degraded components in a fuel mixture (Jordan and Payne 1980). On the other hand, cycloalkanes particularly resist microbial attack. Indeed, complex alicyclic compounds such as tripentacyclic compounds are among the most persistent components of petroleum spillages (Atlas 1981).

The metabolic pathways for the degradation of asphaltic compounds are the least well understood. The degradation of these sulfur-containing petroleum components has been examined; however, no uniform

degradative pathway has yet emerged even though microorganisms use such compounds (Schwendinger 1968).

It is also known that the qualitative hydrocarbon content of the petroleum mixture influences the degradability of individual hydrocarbon components. As an example, Atlas (1981) observed far less degradation of #6 fuel oil than of a light oil such as #2. Despite this fact, many normally recalcitrant compounds degrade in petroleum mixtures because petroleum, with its multitude of substrates, provides an excellent environment for cooxidation to occur (Atlas 1981).

The nature of the microbes that degrade petroleum has warranted some study. A wide variety of microbes capable of degrading petroleum has been found. Psychrotrophic, mesophilic, and thermophilic microorganisms using petroleum hydrocarbons have been isolated (Atlas 1981). Organisms using petroleum hydrocarbons are widely distributed in marine, freshwater, and soil habitats; the primary factor in their effectiveness, however, seems to be the availability of oxygen, as shown by Hambrick et al. (1980) for estuarine sediments.

The literature confuses the issue of actual numbers of hydrocarbon utiliziers because of methodological differences used in the measurement. Other differences result from the individual activity of the microbes. As an example, pseudomonas could not use naphthalene in the solid form but could use naphthalene dissolved in another hydrocarbon (Atlas 1981).

Depending on the particular hydrocarbon, hydrocarbon degradation can occur over a wide temperature range. The low-temperature inhibition is not due solely to lower microbial activity. Low temperatures retard the rate of volatilization of low-molecular-weight hydrocarbons, many of which are toxic to the microorganisms. Thus, the biodegradation of crude oils was found to be highly dependent on composition and incubation temperature. The difference between microbial activity at 10° and at 20° C was considerable because of low abiotic loss of volatiles at low temperature (Atlas 1981).

4.3 SEQUENTIAL ANAEROBIC/AEROBIC DEGRADATION

The literature generally agrees that anaerobic microorganisms are more effective in performing the initial transformation of compounds such as TCE and PCE. Unfortunately, compounds such as the DCEs and VC, which are more mobile and more toxic, accumulate in such transformation. The latter compounds are generally more easily transformed aerobically, suggesting the possibility of using a sequential anaerobic/aerobic treatment system (Bosma et al. 1989).

Since less chlorinated compounds are more easily degraded aerobically in situ, using anaerobic microbes in the source area and aerobic microbes downgradient seems advantageous (Vogel et al. 1989) and indeed has been accomplished with laboratory reactor systems. Dooley-Danna et al. (1989) considered this process with respect to TCE, but it was also proposed for chloranilines (Kuhn and Sulfito 1989). In the first step aniline or lesser halogenated compounds accumulate. The complete oxidation of the latter compounds occurs during the second stage, where oxygen serves both as a coreactant and as an electron acceptor for the microorganisms.

4.4 UNRESOLVED ISSUES

Clearly, many fundamental unresolved issues persist concerning biotransformations in the environment. Many of these issues stem from the poor reproducibility of biodegradation tests (Howard et al. 1986). Others are due simply to an inability to generalize complicated natural systems. For example, microbial activity is generally believed to lead to less toxic compounds, but instances exist of "microbial activation" (Buhler and Williams 1988) to a more toxic form. The following sections discuss several other broad areas for which it has not been possible to develop predictive capability.

4.4.1 Microbial Activity

Hickman and Novak (1989a,b) stated that the inability to accurately quantify the active microbial biomass in soil and groundwater systems limits our ability to predict subsurface biodegradation. For example, these authors were unable to demonstrate a good correlation between reaction rates and microbial density. Such a finding agrees with that of Borden et al. (1986), who stated that, "in many field situations, large variations in microbial population and growth kinetics have little effect on contaminant distributions," probably because microbial growth is so much faster than the water flow and equilibrium is reached very quickly (Srinivasan and Mercer 1988). Furthermore, the highest concentration of microbes may not coincide with the highest concentrations of contaminants. For example, higher organism counts are typically found in the sandy zones of a soil (Looney and Marine 1986), while the contaminants may concentrate in the more fine-grained portions of the aquifer (Wilson and Conrad 1986, J. L. Wilson 1989).

Determining if the groundwater conditions will promote biodegradation is hampered by the need to study the subsurface by means of a well. Groundwater microbes may not be representative of the soil, and monitor wells may interfere with attempts to study the organisms (Thomas et al. 1987). The well provides a continuous source of dissolved oxygen (DO) and may alter redox conditions. Well structures may provide surfaces for microbial colonization, and attached microorganisms may slough into the water pumped from the formation during sample collection. Groundwater flow from the formation into the well during pumping may be faster than the movement of the subsurface microorganisms. Thus, microbial numbers, diversity, and metabolic potential around the well may not reflect the formation. For these reasons, Thomas et al. (1987) suggested that the well should be treated with a short-lived oxidizing material such as hydrogen peroxide before collecting a sample so that microflora representative of the formation will be collected, although numbers and activity may be less.

Many authors are starting to look at ways in which microbial activity can be estimated at a site. Lindgaard-Jorgensen (1988) presented a generalized testing idea to predict degradability with little or no information. The technique seems best suited, however, for heavier petroleum hydrocarbons. Finally, the relative activity of surface soils and groundwater also needs further study. The amount of bacteria in groundwaters is known to be less than in surface soils, but it is still apparently adequate for significant biodegradation (Ventullo and Larson 1985).

4.4.2 Compound-Specific Effects

Biodegradation reactions are influenced both by the specific compounds and by the effects of other compounds. Such compound-specific reactions are difficult to predict. As noted, *o*-xylene is reportedly more difficult to degrade than the *p*-xylene or *m*-xylene isomers. Other authors, however, report that meta-substituted compounds are more difficult to degrade (Alexander and Lustigman 1966).

The effects of cocontaminants are only beginning to be recognized (Scholz-Muramatsu et al. 1988). For example, *cis*- and *trans*-1,2-DCE, 1,1-DCE, and PCE inhibit the degradation of TCE (Eng et al. 1988, Garland et al. 1989). Indeed, the effect of two carbon sources is exceedingly complex. When two carbon sources are available in high concentrations, bacteria in a pure culture metabolize whichever source supports the highest growth rate. If a deficiency exists, however, the bacteria will consume both compounds at once (Zaidl et al. 1988). And not all effects are negative. Nelson et al. (1989) and Cline and Viste (1984) report that aromatics can stimulate the degradation of TCE; unfortunately, this is apparently not true in all circumstances. Wilson et al. (1987) reported extensive biodegradation of aromatics at a fuel spill but found that TCE was not degraded at the site.

Reports note similar effects for single compounds. For example,

low phenol concentrations can stimulate biodegradation and provide a sole-carbon source (Portier et al. 1983), but greater phenol concentrations can poison the organisms and inhibit or prevent biodegradation (Wiggins and Alexander 1988). Kerr and Capone (1988) studied polynuclear aromatic hydrocarbons and also reported that the concentration of the compound was most important--even more important than rather large salinity differences in the system.

The biodegradability of broad classes of compounds and the possibility of determining biodegradability before exhaustive testing are also being studied. Walton and Anderson (1988) tried predicting biodegradability by structural properties with limited success: predictability is excellent within a homologous series but not between heterogeneous series. Similarly, Boethling and Sabijic (1989) believe chemical structure may account for nearly 90% of the variance in biodegradability estimates for 46 diverse compounds. These authors also reported unexplained exceptions: for example, 2-methyl naphthalene is much more persistent than naphthalene.

4.4.3 Solution Effects

Organic content, redox potential, Do, and plate counts may all be needed to estimate biodegradation capabilities of a media (Cline and Viste 1984). The effect of pH may be important because uptake for many organisms is optimum at pH 6 and may be a factor of 10 lower at pH 3 (Albrechtson 1989).

Hickman and Novak (1989b) observed that elevation of temperature increases the rate of reaction but also noted that degradation rates varied considerably over small distances, emphasizing the heterogenous nature of microbial activity in soils. At a single concentration salinity had a significant effect (Kerr and Capone 1988) although significant biodegradation occurred over a wide range of salinities.

4.4.4 Interface Effects and Migration of Microbes

The study of hydrocarbon biodegradation requires more focus on interface effects. Microbial degradation occurs primarily at the oil-water interface (Atlas 1981). Indeed, the interactions of microorganisms, oil, and the environment are not completely understood. Furthermore, the saturated and the unsaturated zones may influence both physical features (such as density and solubility) as well as microbial activity (Streile et al. 1987).

4.4.5 Contaminant Availability

Some work with EDB also shows why predictive capabilities are limited. EDB persists in surface soil for many years despite its volatility, water solubility, low water/soil partition coefficient, and the ability of the soil to degrade a freshly added spike. According to Pignatello (1987), "Apparently some EDB penetrates soil intraaggregate micropores from which it diffused into the aqueous phase very slowly and in which it is inaccessible to microbial attack." Indeed, most investigators agree that sorbed chlorinated solvents are impervious to microbial attack (Alvarez et al. 1989, Barrio-Lage et al. 1988, Chang and Rittman 1987).

4.4.6 Acclimation Period

Many authors discussed the acclimation rate or adaptation period for microbial growth (Suflita and Miller 1985), again with confusing results. The adaptation period varied with concentration for some compounds, but for others there was no concentration effect. Similarly, for some compounds the biodegradation rate changed with concentration, while others showed no effect (Aelion et al. 1989).

4.4.7 Kinetics

The theory of Michaelis and Menton, who applied the general theory of reaction kinetics to enzyme-catalyzed reactions, describes many xtenhematical systems (Lehninger 1970). The extension of Michaelis-Menton kinetics to subsurface porous media biodegradation processes has not been extensively studied. Perhaps uptake kinetics are slowed by accumulations of biomass or limited by slow diffusion through pore fluid. Siegrist and Gujer (1985) included diffusion limitations in their subsurface uptake models by using the theory of biofilms. Unfortunately, biofilm theory requires a number of parameters, such as colony size, which are quite difficult to obtain. For these reasons, predicting reaction rates is extremely difficult.

5. BIOREMEDIATION CONCEPTS

5.1 GENERAL

Aerobic bacteria such as pseudomonas are commonly used for in situ contaminant biodegradation. These organisms can potentially convert organic compounds to carbon dioxide and water without producing toxic reaction products (Amdurer et al. 1985). This capability has led to several attempts to develop more-effective methods of using these microorganisms.

When designing microbial waste treatment systems, one must determine oxygen and nutrient requirements, maintaining appropriate concentrations of nitrogen, phosphorus, and trace elements, as well as appropriate pH values. For example, the heterotrophic bacteria responsible for consuming most organic contaminants work best at pH 6 to 8 and may not work at all at pH below 4.5 or above 8 (Flathman et al. 1989). Nutrient concentrations must be determined during the site investigation; even more important is assessing whether subsurface conditions are adequate to permit control of oxygen supply, temperature, and permeability.

5.2 UNSATURATED SYSTEMS

There has been little research performed on in situ degradation in unsaturated systems. Such projects would be dependent on the soil particle size (Lee and Carberry 1989). Indeed, heterogenities and resulting preferential flow paths limit the application of all in situ techniques.

Most work focuses on using soil piles to remove contaminants in the

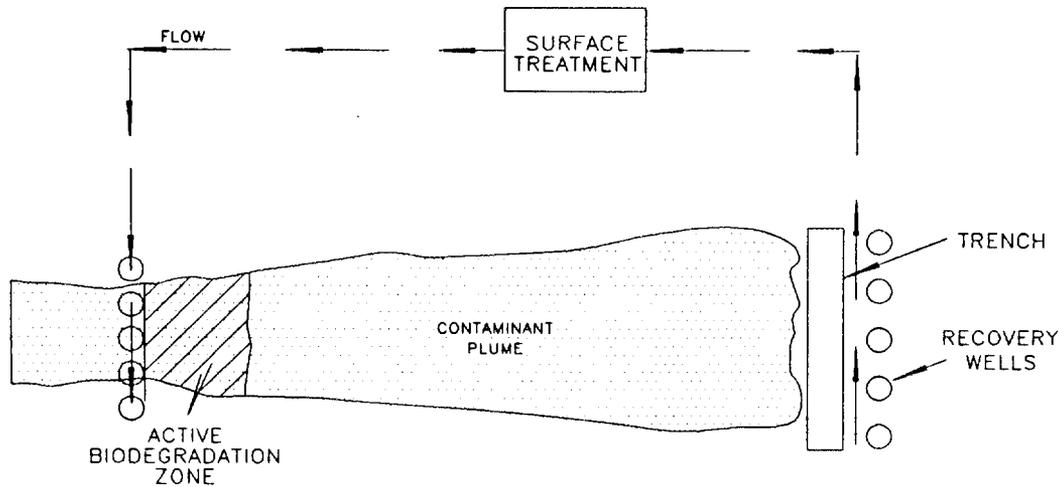
pile or from another source (Kampbell et al. 1987). Wilson and Wilson (1985) and Fogel et al. (1986) studied unsaturated systems and reported complete oxidation of TCE with added methane and oxygen.

As expected, weather conditions can have a significant effect. For example, microbe populations and activity were monitored in an operating petroleum-waste land-treatment facility for 18 months. Seasonal influences were apparent. During the colder, wetter seasons, microbe populations were smaller, less variable and inhibited by the adverse environmental conditions. Hotter, drier months supported large active populations that experienced large variations in numbers and respiratory output. Large variations were also observed within microenvironments of systemic aggregates (Marshall and Deviny 1988).

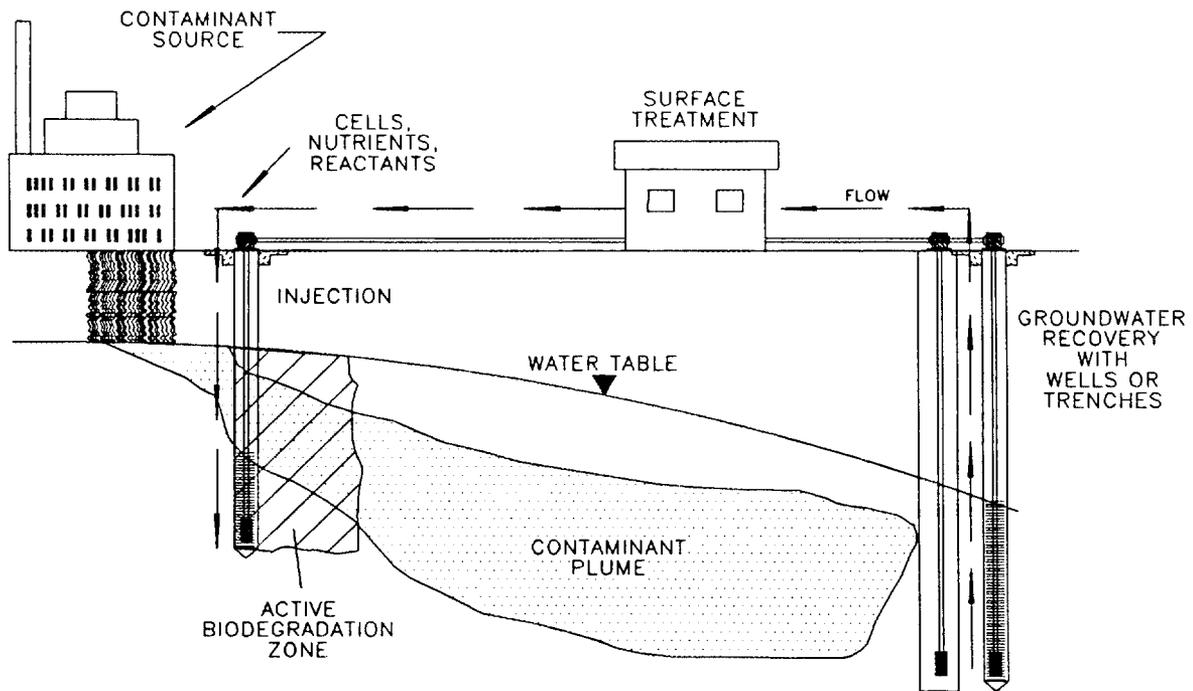
5.3 SATURATED SYSTEMS

A plan view for an idealized in situ biotreatment scheme is shown in Fig. 4. Unfortunately, laboratory studies indicated that clogging of soil pores was likely to be a problem when stimulating aerobic biodegradation in situ (Arvin 1989). Biodegradation is limited by the coefficient of aeration (Rifai et al. 1988); thus, any clogging of soil pores will inhibit the process. Indeed, soil structure seems to have a decisive influence on the potential success of any in situ bioremediation scheme (Werner 1989, Morgan and Watkinson 1989). Field studies demonstrate that clogging is a problem even in highly transmissive strata (Semprini et al. 1987).

Some researchers have used specially prepared microbes rather than merely stimulating indigenous ones. Commercial microbes apparently begin working faster because they need no acclimation period (Bianchini et al. 1988). Lee et al. (1988), however, reported that commercial microbes are not always successful because the inoculated organism may consume the wrong organic compound.



PLAN VIEW



CROSS SECTION

KC713

Fig. 4. Plan view for idealized in situ biotreatment system (Cunningham et al. 1988a).

Furthermore, biotreatment may not remove all of the contaminant. Even quite volatile compounds may penetrate the soil and be unavailable for biodegradation (Pignatello 1987). As an example, studies have been reported showing that biostimulation removes contamination from the water, but, as soil cores taken later show, not from the soil (Lee and Ward 1985). Two other sources (International Technology 1986, Wilson and Brown 1989) claim nearly complete removal, but neither described how the removal was quantified. This inability to remove contaminants from the soil was demonstrated in one field study in which flushing circuits supplied materials to the subsurface to optimize bacterial growth. Good water quality was quickly achieved, but unfortunately the soil was still highly contaminated. Biodegradable surfactants were then added to improve flushing and dissolve more hydrocarbon. The technique worked, except that the organisms also metabolized the surfactants and the resulting increase in biomass quickly plugged the system. Experiments are now continuing to determine whether inorganic surfactants or flocculating agents such as sodium pyrophosphate enhance the availability of the hydrocarbons without reducing the system's permeability (Werner 1989).

J. T. Wilson (1989) quantitatively addressed the magnitude of the error possible in bench studies by showing that laboratory experiments designed to mimic field conditions yielded 20% biodegradation per day, while actual field work yielded only 0.3%. The conclusion was that heterogeneity was the controlling factor and that a field estimate of reaeration capacity was needed to predict what would happen at a particular site.

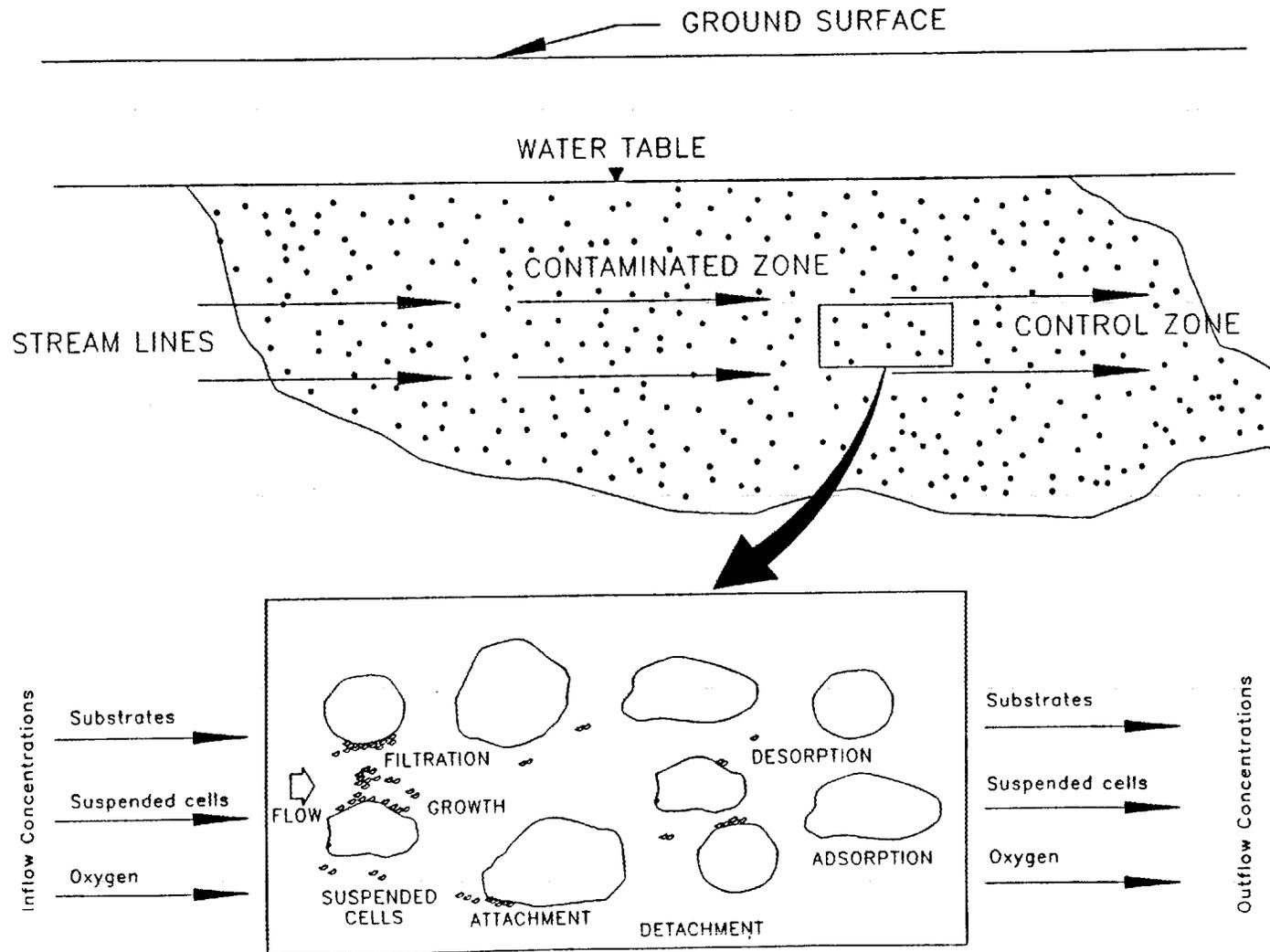
As noted, the coefficient of reaeration or the ability of the system to supply oxygen determines how well bioremediation continues in situ. Because of the difficulty of supplying oxygen and because of its low solubility in water, experiments have been performed in which hydrogen peroxide has been injected into the aquifer (American Petroleum Institute 1982). Other techniques that have been explored include using

an aboveground bioreactor to first remove free products and to metabolize dissolved hydrocarbon. The treated water, now seeded with microorganisms and oxygen, is then reinjected to continue treatment in situ (Von Wedel et al. 1988).

In situ remediation depends on the development of a biofilm on the surface of soil particles. Thus, there is no biotransformation if the contaminants are adsorbed as noted previously for laboratory studies. This effect has also been demonstrated in field studies (Semprini et al. 1989). Since most of the microorganisms are in the biofilms, the contaminants, as well as all necessary nutrients, must be contained in the flowing groundwater for the organisms to react with them. However, microbes do move in the soil and move further than might be expected. A better understanding of the factors influencing deposition and transport of microbes transforming organic contaminants in porous media will aid the development of in situ treatment technology (Cunningham et al. 1988a). Some general equations have been developed which show, for example, why TCE must be cometabolized and not used as a sole-carbon source (Cunningham et al. 1988b).

5.4 MODELING

A review of the literature clearly indicates that numerical models have limited applicability for predicting the rate and extent of biodegradation in either the saturated or the unsaturated zones. The reason for the limited applicability is that the system is so complex (see Fig. 5) that the large number of variables creates the need to solve a nonlinear problem. The situation is similar to solving a single equation with two or more unknowns. The system is even more complicated because it involves mixed media. For example, the microorganisms reside in a fixed biofilm and interact with dissolved and solid particles flowing past the biofilm. Indeed, the biofilm processes occurring in



KC716

Fig. 5. Illustration of the numerous variables involved with modeling biodegradation (modified from Cunningham et al. 1988a).

porous media flow are more difficult to monitor than those occurring in other common reactor types such as tanks or reservoirs.

Subsurface biofilm accumulation reacts to fluid and nutrient transport, which in a porous media occurs along tortuous flow paths of varying dimension and geometry. Similarly, the wide distribution of pore velocities introduces considerable variation in adsorption, desorption, attachment and detachment. Filtration mechanisms depositing particulate matter must also be considered. An understanding of cause-and-effect relationships influencing these biofilm processes is essential if net subsurface biofilm accumulation is to be adequately described. Another modeling item that must be addressed is analysis and mitigation of biofouling (Cunningham et al. 1988b).

Cunningham is one of few authors attempting to model in situ microbial action (Cunningham et al. 1988b). Baek et al. (1989) showed that, from the aspect of hydraulics, biomass buildup in a soil system could be described by an increase in silt content. The modeling showed that increasing soil biomass and distributing the soil microorganisms throughout the soil depth improved removal of a contaminant. Furthermore, infrequent application cycles subdue excessive growth of soil microbes that clog the system. The work of Baek et al. (1989) represents one of the few attempts to model biodegradation in the unsaturated zone. These investigators used a model named BIOSOIL to study the influence of microorganisms on soil water flow and chemical removal rates. Their investigation provided some insight into biomass buildup and the importance of soil depth; the model, however, is not developed sufficiently to allow field calibration.

More work has been performed in modeling biodegradation in the saturated zone. A review of the literature, however, demonstrates that additional development of predictive models is hampered by two major obstacles. First, as noted previously, the systems are too complex, and are subject to several interdependent factors affecting biodegradation. Second, measuring or reliably estimating the subsurface parameters that

control biodegradation remains very difficult.

Rifai et. al. (1988) refined previous efforts of Borden and Bedient (1988) to model biodegradation in an oxygen-limited groundwater system. Adsorption, biomass distribution, biofilm accumulation, and anaerobic biodegradation were all assumed to be simulated by the coefficient of reaeration and the oxygen distribution in the system. Moreover, the investigators also assumed instantaneous reaction rates and groundwater mixing, assumptions which are all questionable; the resulting model is more empirical than quantitative.

Srinivasan and Mercer (1988) improved the simulation of organics sorption by using linear equilibrium isotherms. Once again, however, their model solved a nonlinear problem by using estimates of several controlling parameters. This lack of fundamental information has been discussed by Kindred and Celia (1989), who state that successful modeling requires an improved understanding of the metabolic pathways dominating the particular setting, along with improved estimates of kinetic parameters. The result of so many estimates and assumptions is that available models are applicable only to a specific site and reliable only during the time in which the site is being monitored.

Because of the complexity of subsurface systems and the number of factors involved, the best hope for predicting biodegradation appears to be case histories. By selecting a site with similar hydrogeology, similar composition, similar concentrations of contaminants, and a long history of monitoring, empirical relationships might be developed to predict the rate of biodegradation at that site.

6. COMMERCIAL PROCESSES

It is apparent from the foregoing that bioremediation of soils and groundwater contaminated with hazardous waste is a field in its infancy. Research groups working on various aspects of the problem for approximately 10 years continue to report contradictory results despite general agreement on broad aspects. To evaluate the claims and counter-claims of various commercial vendors, several vendors were contacted in the fall of 1989 concerning their bioremediation experience.

6.1 COMPANY A

The information in this section was acquired from the company's vice president and from some of the company's marketing literature. Company A has recently completed an in situ pilot study on TCE removal from groundwater at a California site. They seeded the site with *Pseudomonas G-4* and a proprietary compound that would oxygenate and provide a carbon source for the microorganisms without stimulating too much biomass. This particular organism effectively degrades phenol and produces an enzyme that degrades chlorinated solvents. The depth to water at the site was 130 ft, and the TCE in one location was reduced from 3000 ppb to less than 50 ppb within 5 d.

Based on the literature review in this report, however, several issues must be resolved before this pilot study can be called an unqualified success:

1. Others have successfully cleaned up groundwater (although not TCE) in situ, only to find that sorbed or physically occluded contaminants soon recontaminated the water.

2. Aquifer heterogeneity usually prevents contact with all of the contamination.
3. The test was quite short--perhaps too short to see a clogging problem from buildup of biomass.

Company A personnel were also questioned concerning how they would treat contamination in the vadose zone. They stated that they have flooded the soil with water and nutrients but added that this technique has several problems, the most basic of which is confining the nutrient solution to keep it from affecting and recontaminating the groundwater.

Company A personnel also suggested that bioremediation might not be practical for shallow contaminated soils in the vadose zone and suggested in situ vacuum extraction.

Finally, when asked about success with 1,1,1-TCA, Company A personnel stated that they had concentrated on TCE and had varying success with other solvents.

6.2 COMPANY B

This company has recently published several articles reporting laboratory studies with bioreclamation techniques. The information in this section is from a conversation with the company's director of bioremediation. Company B's experience with remediation of the shallow unsaturated zone has been primarily with in situ vacuum extraction. Company B found bioreclamation much more difficult in clay soils and with solvent and petroleum mixtures. The research director indicated that in situ bioreclamation techniques are too experimental and that she needed to "dig it (waste) up." This contrasts with the company's brochure, which describes a method for treating contaminated permeable soil above the water table with in situ biodegradation. Forced aeration delivers oxygen, and a mineral nutrient solution is added by percolation

from the surface. Based on the literature reviewed, this technique may reduce contaminant levels but may not be adequately effective. This conclusion is based on the obvious inability to control where the oxygen and nutrient solutions flow.

The company has experience with aboveground soil pile arrangements. Not much of a pilot study is needed. Soil from the site is taken to the laboratory in order to learn how to stimulate the indigenous microorganisms. If indigenous microorganisms are absent, other microorganisms may be added. The system consists of a soil pile to which a mineral nutrient solution is added, along with forced aeration through a network of air ducts.

6.3 COMPANY C

Company C is an experienced environmental remediation firm. Taking the posture that no one had successfully cleaned up chlorinated solvents by using aerobic biodegradation in the field, this group felt that fuel hydrocarbons could be managed by aerobic biodegradation but suggested consultation with some of the university research groups working in this field.

6.4 COMPANY D

Company D has no experience with bioremediation in soils. Its process has been used to clean up sludges in lagoons, where biodegradation can take place at the sludge/water interface where water supplies oxygen for the microorganisms. They recommend ponding contaminated soil and treating the soil in the impoundment.

6.5 COMPANY E

This company performs no field work and confines itself to pilot studies, site reviews, and selection and review of the work of remedial action contractors. As such, the company has no vested interest in who performs the remediation work.

Company E personnel advocate infiltration galleries (described previously by Company B) for cleaning up petroleum hydrocarbons in the unsaturated zone but are skeptical about using this technique for chlorinated solvents. In their view, compounds such as TCE and 1,1,1-TCA partition more in the soil and are often missed by the infiltrating solution. Furthermore, the initial degradation products are more soluble, making it difficult to keep from washing them along with the parent compound into the saturated zone.

They were also skeptical about seeding with bacterial cultures, in direct contrast to the many companies who focus on their own proprietary cultures. Personnel at Company E believe in stimulating the indigenous bacteria because no proof exists that the seeded bacteria will work at a particular site until the experiment is actually performed in situ.

6.6 COMPANY F

This company has a lengthy list of references for its biotreatment experience. Most of the company's work is with fuel spills, but personnel claim some experience with chlorinated solvents. Typically, with shallow contamination, Company F uses an aboveground treatment cell. Employees seed the soil with the company's own bacteria, add water and nutrients, and continually monitor the process. Soil cleanup generally takes less than 90 d, and the soil can be released as fill dirt when the project is completed.

6.7 COMPANY G

This company has never performed any in situ projects but is experimenting with them. Company personnel prefer to perform treatment aboveground because it ensures better control and adequate mixing. They also believe aboveground treatment will be less expensive than in situ techniques because the control that can be exercised over the process results in complete cleanup in a relatively predictable time period-- something they believe will not be achieved in situ.

Company G uses indigenous microorganisms. Brief laboratory studies identify and acclimate the organisms, which are inoculated into rectangular treatment bays about 2 ft deep. The soil is monitored so that moisture and nutrient needs are satisfied. A Rototiller periodically mixes the soil and nutrients.

6.8 COMPANY H

This company claims to have successfully treated vadose zone contaminants in situ. Having made this statement, however, the contact said that delivery of the nutrients often cannot be adequately controlled for in situ processes. Furthermore, he felt that in situ techniques would not work very well if there is clay in the soil. He also felt that aboveground systems were much easier to permit and were often cheaper (estimated costs \$50-\$100 per yard). This company also prefers stimulating the indigenous bacteria and feels that a pilot study is unnecessary.

Company H uses an aboveground "treatment cell." The cell is ventilated and used for nutrient enhancement.

6.9 COMPANY I

This company requested site-specific information before it would provide any definite answers. Company personnel did believe that aboveground treatment was much more effective than in situ. They use their own bacteria, after pilot testing to develop the best bacteria mixture and nutrient requirements.

The marketing director who supplied this information reported good success breaking down PCBs, a claim which seems overstated. Although PCBs degrade microbially, extensive studies have shown that degradation is slow and somewhat unpredictable (Portier and Fujasaki 1988, Abramowicz et al. 1989). In a follow-up telephone call, the marketing director admitted that the company was looking for a place to perform a pilot study for PCB biodegradation and had no field experience.

6.10 SUMMARY OF INFORMATION FROM COMMERCIAL VENDORS

Generally, none of the commercial vendors expressed confidence about using in situ techniques. Most said they could or would do it, but all preferred to discuss how they accomplish bioreclamation aboveground.

Furthermore, controversy exists about the need for proprietary microorganisms, a controversy which carries over from the technical literature reviewed in this report. No one demonstrated any quantitative advantage for proprietary organisms. Indeed, the review article by Lee et al. (1988) states reasons why introduced microorganisms often do not work.

Finally, intermittent operation would be a problem. All aboveground treatment scenarios use soil piles that are treated as a unit. All soil is disposed of, once treatment is complete. While acceptable for shallow soils, aboveground treatment might not be

appropriate for contaminated soils discovered during deeper excavation. These soils would require stockpiling until enough soil was obtained to make treatment feasible.

6.11 TREATMENT COSTS

Little information in the technical literature concerns treatment costs. Apparently, however, aboveground bioremediation of very large quantities of soils (e.g., 3000 yd³) costs from \$50 to \$80 per yard (Torpy et al. 1989). Smaller projects (e.g., 200 yd³) could cost two to three times as much.

7. CONCLUSIONS

This report presents a number of important findings concerning degradation mechanisms for chlorinated solvents and petroleum hydrocarbons. These findings include the following:

- Chemical degradation is typically not important. A potential exception, however, is dehydrohalogenation of compounds such as 1,1,1-TCA at warm sites low in organic matter.
- Anaerobic biodegradation of TCE may form either 1,1-DCE, cis-1,2-DCE, or trans-1,2-DCE and eventually may form chloroethene (vinyl chloride). Research has not quantified the conditions under which formation of one isomer is favored over another.
- Petroleum hydrocarbons degrade most easily under aerobic conditions. Additional branching and substitution generally lead to lower rates of degradation.

The report also addresses the potential role of bioreclamation in remediating soils and groundwater contaminated with chlorinated solvents and petroleum hydrocarbons. Important findings include the following:

- Many researchers and commercial vendors are pessimistic concerning in situ techniques because of the difficulty of overcoming heterogeneities in the soil.
- Success has been achieved with aboveground bioreclamation of soils contaminated with petroleum hydrocarbons.
- Bioreclamation of soil and water contaminated with chlorinated solvents is an area of active research. The most promising techniques employ methanotrophic bacteria in aboveground bioreactors.

REFERENCES

- Abramowicz, D. A., M. J. Brennan, and H. VanDort. 1989. Anaerobic biodegradation of polychlorinated biphenyls. pp. 377-379. IN Proc., 194th American Chemical Society National Meeting, Miami, Florida.
- Aelion, C. M., D. C. Dobbins, and F. K. Pfaender. 1989. Adaptation of aquifer microbial communities to the biodegradation of xenobiotic compounds: Influence of substrate concentration and preexposure. Environ. Toxicol. Chem. 8:75-86.
- Albrechtsen, H. 1989. Influence of environmental factors on microbial biomass and activity in aquifers. Paper presented at the International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, California.
- Alexander, M., and B. K. Lustigman. 1966. Effect of chemical structure on microbial degradation of substituted benzenes. J. Agric. Food Chem. 14:410-413.
- Alvarez, L. M., P. L. McCarty, and P. V. Roberts. 1989. The effects of sorption on the biotransformation rate of TCE by methanotrophs. Paper presented at the International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, California.
- Amdurer, M., R. Fellman, and S. Abdelhamid. 1985. In-situ treatment technologies and Superfund. International Conference on New Frontiers for Hazardous Waste Management. EPA/600/9-85/025. U.S. Environmental Protection Agency.

- American Petroleum Institute. 1982. Enhancing the microbial degradation of underground gasoline by increasing available oxygen. American Petroleum Institute, Washington, DC.
- Arvin, E. 1989. Kinetics of aerobic biological degradation of chlorinated aliphatic compounds. Paper presented at the International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, California.
- Atlas, R. M. 1981. Microbial degradation of petroleum hydrocarbons: An environmental perspective. *Microb. Rev.* 45:180-209.
- Bae, W., B. E. Rittmann, J. E. Odenrantz, and A. J. Valocchi. 1989. Biodegradation of halogenated solvents by biologically active zones induced by nitrate injection into porous-medium flow: I. Laboratory experiments. Paper presented at the International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, California.
- Baek, N. L., L. S. Clesceri, and N. L. Clesceri. 1989. Modeling of enhanced biodegradation in unsaturated soil zone. *J. Environ. Eng.* 115:150-172.
- Barrio-Lage, G., F. Z. Parsons, R. S. Nassar, and P. A. Lorenzo. 1986. Sequential dehalogenation of chlorinated ethenes. *Environ. Sci. Technol.* 20:96-99.
- Barrio-Lage, G., F. Z. Parsons, R. S. Nassar, and P. A. Lorenzo. 1987a. Biotransformation of trichloroethene in a variety of subsurface materials. *Environ. Toxicol. Chem.* 6:571-578.

- Barrio-Lage, G., F. Z. Parsons, and R. S. Nassar. 1987b. Kinetics of the depletion of trichloroethene. *Environ. Sci. Technol.* 21:366-370.
- Barrio-Lage, G., F. Z. Parsons, and P. A. Lorenzo. 1988. Inhibition and stimulation of trichloroethylene biodegradation in microaerophilic microcosms. *Environ. Toxicol. Chem.* 7:889-895.
- Berry, D. F., A. J. Francis, and J. M. Bollag. 1987. Microbial metabolism of homocyclic and heterocyclic aromatic compounds under anaerobic conditions. *Microbiol. Rev.* 51:43-59.
- Bianchini, M. A., R. J. Portier, K. Fujisaki, C. B. Henry, P. H. Templet, and J. E. Matthews. 1988. Determination of optimal toxicant loading for biological closure of a hazardous waste site, pp. 503-516. IN Special Technical Publications 971. American Society for Testing and Materials, Philadelphia.
- Boethling, R. S. and A. Sabijic. 1989. Screening-level model for aerobic biodegradability based on a survey of expert knowledge, *Environ. Sci. and Technol.* 23:672-679.
- Borden, R. C. and P. B. Bedient. 1986. Transport of dissolved hydrocarbons influenced by reaeration and oxygen limited biodegradation: 1. Theoretical development. *Water Resour. Res.* 22:1973-1982.
- Borden, R. C., P. B. Bedient, M. D. Lee, C. H. Ward, and J. T. Wilson. 1986. Transport of dissolved hydrocarbons influenced by oxygen limited biodegradation: 2. Field application. *Water Resour. Res.* 22:1983-1990.

- Bosma, T. N. P., G. Schrae, and A. J. B. Zehnder. 1989. Biotransformation of chlorinated micropollutants in sediments columns. Paper presented at the International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, California.
- Bouwer, E. J., B. E. Rittmann, and P. L. McCarty. 1981. Anaerobic degradation of halogenated 1- and 2-carbon organic compounds. *Environ. Sci. Technol.* 15:596-599.
- Bouwer, E. J. and P. L. McCarty. 1983. Transformations of 1- and 2-carbon halogenated aliphatic organic compounds under methanogenic conditions. *Appl. Environ. Microbiol.* 45:1286-1294.
- Bouwer, E. J., G. D. Cobb, and D. L. Pardieck. 1989. Effect of electron acceptor on organic contaminant biotransformations, Paper presented at the International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, California.
- Boyd, S. A. 1987. Reductive dechlorination of organic toxicants in anaerobic soils. pp. 411-412. IN Proc., 192nd American Chemical Society National Meeting, Denver, Colorado.
- Buhler, D. R., and D. E. Williams. 1988. The role of biotransformation in the toxicity of chemicals. *Aquat. Toxicol.* 11:19-28.

- Castro, C. E., and N. O. Belser. 1990. Biodehalogenation: Oxidative and reductive metabolism of 1,1,2-trichloroethane by *Pseudomonas putida*-biogeneration of vinyl chloride. *Environ. Toxicol. Chem.* 9:707-714.
- Chang, H. T., and B. E. Rittman. 1987. Bioavailability of sorbed organic compounds. pp. 403-405. IN Proc., 192nd American Chemical Society National Meeting, Denver, Colorado.
- Cline, P. V., and D. R. Viste. 1984. Migration and degradation patterns of volatile organic compounds. *Waste Manage. Res.* 3:351-360.
- Cline, P. V., J. J. Delfino, and W. J. Cooper. 1986. Hydrolysis of 1,1,1-trichloroethane; formation of 1,1-dichloroethene. IN Proceedings of the NWWA/API Conference on Petroleum Hydrocarbons and Organic Chemicals in Ground Water, Houston, Texas, 1986. National Water Well Association, Dublin, Ohio.
- Cooper, W. J., M. Mehran, D. J. Riusech, and J. A. Joens. 1987. Abiotic transformations of halogenated organics: 1. Elimination reaction of 1,1,2,2-tetrachloroethane and formation of 1,1,2-trichloroethene. *Environ. Sci Technol.* 21:1112-1114.
- Cunningham, A. B., W. G. Characklis, and E. J. Bouwer. 1988a. In-situ control of groundwater contaminants by microbial processes. U.S. Geologic Survey Project 14-08-0001-G1284.
- Cunningham, A. B., D. J. Crawford, and W. G. Characklis. 1988b. Modeling microbial transport in porous media. *Water Sci. Technol.* 20:509-511.

- Curtis, G. P., and M. Reinhard. 1989. Reductive dehalogenation of hexachloroethane by aquifer materials. Paper presented at the International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, California.
- Dooley-Danna, M., S. Fogel, and M. Findlay. 1989. The sequential anaerobic/aerobic biodegradation of chlorinated ethenes in an aquifer simulator. Paper presented at the International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, California.
- Eng, W., A. V. Palumbo, and C. D. Little. 1988. Kinetics of trichloroethylene degradation by a methane utilizing bacterium under oxygen-limiting conditions. IN Proceedings of the 88th Annual Meeting, American Society for Microbiology, Miami, Florida.
- Flathman, P. E., D. E. Jerger, and L. S. Bottomley. 1989. Remediation of contaminated groundwater using biological techniques. Groundwater Monit. Rev. Winter:105-119.
- Fogel, M. M., A. R. Taddeo, and S. Fogel. 1986. Biodegradation of chlorinated ethenes by a methane-utilizing mixed culture. Appl. Environ. Microbiol. 51:720-724.
- Garland, S. B., A. V. Palumbo, G. W. Strandberg, T. L. Donaldson, L. L. Farr, W. Eng., and C. D. Little. 1989. The use of methanotrophic bacteria for the treatment of groundwater contaminated with trichloroethene at the U.S. Department of Energy Kansas City Plant. ORNL/TM-11084.

- Goldsmith, C. D., Jr., and R. K. Balderson. 1988. Biodegradation and growth kinetics of enrichment isolates on benzene, toluene and xylene. *Water Sci. Technol.* 20:505-507.
- Hambrick, G. A., R. D. DeLaune, and W. H. Patrick. 1980. Effect of estuarine sediment pH and oxidation-reduction potential on microbial hydrocarbon degradation. *Appl. Environ. Microbiol.* 40:365.
- Hanson, R. S., G. A. Brusseau, and L. P. Wackett. 1989. Development of methanotrophs for the biodegradation of trichloroethylene and other chlorinated olefins. pp. 365-367. IN Proc., 194th American Chemical Society National Meeting, Miami.
- Henry, S. M., D. Grbic-Galic. 1989. Effects of availability of reducing power on TCE transformation by methanotrophs. Paper present at the International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, California.
- Hickman, G. T., and J. T. Novak. 1989a. Subsurface biotransformation of organic contaminants under different metabolic conditions. Paper presented at the International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, California.
- Hickman, G. T., and J. T. Novak. 1989b. Relationship between subsurface biodegradation rates and microbial density. *Environ. Sci. Technol.* 23:525-532.
- Howard, P. H., A. E. Hueber, and R. S. Boethling. 1986. Biodegradation data evaluation for structure/biodegradability relations. *Environ. Toxicol. Chem.* 6:1-10.

- Hutchins, S. R., and J. T. Wilson. 1989. Evaluation of denitrification for bioremediation of an aquifer contaminated with JP-4 jet fuel. Paper presented at the International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, California.
- Inman, J. C., S. D. Strachan, L. E. Sommers, D. W. Nelson. 1984. The decomposition of phthalate esters in soil. *J. Environ. Sci. Health* 19:245-251.
- International Technology Corporation 1986. "In-situ bioremediation: Gasoline contamination in southern California. International Technology, Corporation.
- Jaffe, P. R., and N. H. Baek. 1989. Estimating concentrations of trichloroethylene and its intermediates in methanogenic aquifers. Paper presented at the International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, California.
- Jafvert, C. T., and N. L. Wolfe. 1987. Degradation of selected halogenated ethanes in anoxic sediment-water systems. *Environ. Toxicol. Chem.* 6:827-837.
- Jordan, R. E., and J. R. Payne. 1980. *Fate and Weathering of Petroleum Spills in the Marine Environment*. Ann Arbor Science Publishers, Ann Arbor, Michigan.
- Kampbell, D.H., J. T. Wilson, H. W. Read, and T. T. Stocksdale. 1987. Removal of volatile aliphatic hydrocarbons in a soil bioreactor. *J. Air Pollut. Control Assoc.* 37:1236-1240.

- Kerr, R. P., and D. G. Capone. 1988. The effect of salinity on the microbial mineralization of two polycyclic aromatic hydrocarbons in estuarine sediments. *Mar. Environ. Res.* 26:181-198.
- Kindred, J. S. and M. A. Celia. 1989. Contaminant transport and biodegradation: 2. Conceptual model and test simulations. *Water Resour. Res.* 25:1149-1159.
- Kleopfer, R. D., D. M. Easley, B. B. Haas, Jr., and T. G. Delhi. 1985. Anaerobic degradation of trichloroethylene in soil. *Environ. Sci. Technol.* 19:277-280.
- Kuhn, E.P., P. J. Colberg, J. L. Schnoor, O. Wanner, A. J. B. Zehnder, and R. P. Schwarzenbach. 1985. Microbial transformations of substituted benzenes during infiltration of river water to groundwater: Laboratory column studies. *Environ. Sci. Technol.* 19:961-968.
- Kuhn, E. P., and J. M. Suflita. 1989. Sequential reductive dehalogenation of chloroanilines by microorganisms from a methanogenic aquifer. *Environ. Sci. Technol.* 23:848-852.
- Lee, M. D., J. M. Thomas, R. C. Borden, P. B. Bedient, J. T. Wilson, and C. H. Ward. 1988. Bioremediation of aquifers contaminated with organic compounds. *CRC Crit. Rev. Environ. Control* 18:29-89.
- Lee, M. D., and C. H. Ward. 1985. Biological methods for the restoration of contaminated aquifers. *Environ. Toxicol. Chem.* 4:743-750.

- Lee, S. H., and J. B. Carberry. 1989. Fate and transport of petroleum contaminants under biotic and abiotic conditions in the unsaturated zone. Paper presented at the International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, California.
- Lehninger, A. L. 1970. Biochemistry. Worth Publishers Inc., New York.
- Lindgaard-Jorgensen, P. 1988. A strategy for evaluation of the degradability of organic material in complex effluents. *Ambio* 17:398-400.
- Little, C. D. 1987. Stimulation of trichloroethene biodegradation in groundwater samples. Abstract No. Q-105. p. 299. IN Abstracts, Annual Meeting, American Society of Microbiologists, Atlanta, Georgia.
- Little, C. D., A. V. Palumbo, S. E. Herbes, M. E. Lindstrom, R. L. Tyndall, and P. J. Gilmer. 1988. Trichloroethylene biodegradation by pure cultures of a methane-oxidizing bacterium. *Appl. Environ. Microbiol.* 54:951-956.
- Looney, B. B., and I. W. Marine. 1986. Distribution of degrading chlorocarbon solvents in water and soil in a contaminant plume. Paper presented at the Special Symposium on Scientific Advances in Geology and Hydrology from Studies of Groundwater Plumes, 1986 Geological Society of America Meeting, San Antonio, Texas.
- Marshall, T. R. and J. S. Devinny. 1988. The microbial ecosystem in petroleum waste land treatment. *Water Sci. Technol.* 20:285-291.
- Maskarinec, M. P., C. K. Bayne, L. H. Johnson, S. K. Halladay, and R. A. Jenkins. 1989. Stability of volatile organics in environmental water samples: Storage and preservation. ORNL/TM-11300.

- Mayer, K. P., and D. Grbic-Galic. 1989. TCE degradation by methanotrophic bacterial communities in aquifer-simulating microcosms. Paper presented at the International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, California.
- Mirsatari, S. G., M. M. McChesney, and A. C. Craigmill. 1987. Anaerobic microbial dechlorination: An approach to on-site treatment of toxaphene-contaminated soil. *J. Environ. Sci. Health* 22:663-690.
- Moore, A. T., A. Vira, and S. Fogel. 1989. Biodegradation of trans-1,2-dichloroethylene by methane-utilizing bacteria in an aquifer simulator. *Environ. Sci. Technol.* 23:403-406.
- Morgan, P., and R. J. Watkinson. 1989. Assessment of potential for in-situ biotreatment of hydrocarbon-contaminated soils. Paper presented at the International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, California.
- Morrison, R. T., and R. N. Boyd. 1987. *Organic Chemistry*, 5th ed., Allyn and Bacon, Inc., Boston.
- Nelson, M. J. K., D. Ross, and A. W. Bourquin. 1989. Development of biological processes to remediate short-chain chlorinated hydrocarbons. pp. 369-370. IN Proc., 194th American Chemical Society National Meeting, Miami.
- Parsons, F., G. Barrio-Lage, and R. Rice. 1985. Biotransformation of chlorinated organic solvents in static microcosms. *Environ. Toxicol. Chem.* 4:739-742.

- Parsons, F., P. R. Wood, and J. DeMarco. 1984. Transformations of tetrachloroethene and trichloroethene in microcosms and groundwater. *Res. Technol.* 76:56-59.
- Phelps, T. J., D. Ringelberg, D. Hedrick, J. Davis, C. B. Fliermans, and D. C. White. 1988. Microbial biomass and activities associated with subsurface environments contaminated with chlorinated hydrocarbons. *Geomicrobiol. J.* 6:157-170.
- Pignatello, J. J. 1987. Microbial degradation of 1,2-dibromoethane in shallow aquifer materials. *J. Environ. Qual.* 16:307-312.
- Portier, R. J., H. M. Chen, and S. P. Meyers. 1983. Environmental effect and fate of selected phenols in aquatic ecosystems using microcosm approaches. *Dev. Ind. Microbiol.* 24:409-424.
- Portier, R. J. and K. Fujisaki. 1988. Enhanced biotransformation and biodegradation of polychlorinated biphenyls in the presence of aminopolysaccharides. pp. 517-527. IN Special Testing Publication 971. American Society for Testing and Materials, Philadelphia.
- Portier, R. J., and S. I. Ahmed. 1989. A marine biotechnological approach for coastal estuarine site remediation and pollution control. *MTS J.* 22:6-14.
- Reed, J. P., A. J. Keaster, R. J. Kremer, and D. H. Kerr. 1989. Microbial degradation of some soil-applied insecticides, herbicides, and insecticide-herbicide combinations. *Bull. Environ. Contam. Toxicol.* 42:676-681.

- Reinhard, M., N. L. Goodman, and J. F. Barker. 1984. Occurrence and distribution of organic chemicals, in two landfill leachate plumes. *Environ. Sci. Technol.* 18:953-961.
- Richards, D. J., and W. K. Shieh. 1986. Biological fate of organic priority pollutants in the aquatic environment. *Water Res.* 20:1077-1090.
- Rifai, H. S., P. B. Bedient, J. T. Wilson, K. M. Miller, and J. M. Armstrong. 1988. Biodegradation modeling at aviation fuel spill sites. *J. Environ. Eng.* 114:1007-1029.
- Schraa, G., T. N. P. Bosma, C. Holliger, J. R. van der Meer, A. R. W. Van Neerven, M. E. Tros, and A. J. B. Zehnder. 1989. Microbial degradation of chlorobenzenes at low concentrations. Paper presented at the International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, California.
- Schwarzenbach, R. P., W. Giger, C. Schaffner, and O. Wanner. 1985. Groundwater contamination by volatile halogenated alkenes: Abiotic formation of volatile sulfur compounds under anaerobic conditions. *Environ. Sci. Technol.* 19:322-327.
- Schwendinger, R. B. 1968. Reclamation of soil contaminated with oil. *J. Inst. Pet.* 54:182-197.
- Scholz-Muramatsu, H., V. Schneider, S. Gaiser, and D. Bardtke. 1988. Biological elimination of dichloromethane from contaminated groundwater-interference by components of the groundwater. *Water Sci. Technol.* 20:393-397.

- Semprini, L., P. V. Roberts, G. D. Hopkins, and D. M. MacKay. 1987. A field evaluation of in-situ biodegradation for aquifer restoration. EPA/600/2-87/096. U.S. Environmental Protection Agency.
- Semprini, L., G. D. Hopkins, P. V. Roberts, and P. McCarty. 1989. Field studies and model simulations of halogenated aliphatic decomposition by methanotrophic bacteria. Paper presented at the International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, California.
- Siegrist, H., and W. Gujer. 1985. Mass transfer mechanisms in a heterotrophic biofilm. *Water Resour. Res.* 19:1369-1378.
- Srinivasan, P., and J. W. Mercer. 1988. Simulation of biodegradation and sorption processes in ground water. *Ground Water* 26:475-487.
- Streile, G. P., J. M. Zachara, and J. K. Fedrickson. 1987. Review of intermediate-scale experiments for subsurface microbiology and chemistry. DOE/ER-0383. U.S. Department of Energy.
- Suflita, J. M., and G. D. Miller. 1985. Microbial metabolism of chlorophenolic compounds in groundwater aquifers. *Environ. Toxicol. Chem.* 4:751-758.
- Thomas, J. M., M. D. Lee, and C. H. Ward. 1987. Use of groundwater in assessment of biodegradation potential in the subsurface. *Environ. Toxicol. Chem.* 6:607-614.
- Torpy, M. F., H. F. Stroo, and G. Brubaker. 1989. Biological treatment of hazardous waste. *Pollut. Eng.* 6:80-86.

- Van Dyke, R. A. 1977. Dechlorination mechanisms of chlorinated olefins. *Environ. Health Perspect.* 21:121-124.
- Ventullo, R. M., and R. J. Larson. 1985. Metabolic diversity and activity of heterotrophic bacteria in ground water. *Environ. Toxicol. Chem.* 4:759-771.
- Vogel, T. M., and P. L. McCarty. 1985. Biotransformation of tetrachloroethylene to trichloroethylene, dichloroethylene, vinyl chloride and carbon dioxide under methanogenic conditions. *Appl. Environ. Microbiol.* 49:1080.
- Vogel, T. M., B. H. Fathepure, and H. Selig. 1989. Sequential aerobic/anaerobic degradation of chlorinated organic compounds. Paper presented at the International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, California.
- Von Wedel, R. J., J. F. Mosquera, C. D. Goldsmith, G. R. Hater, A. Wong, T. A. Fox, W. T. Hunt, M. S. Paules, J. M. Quiros, and J. W. Wiegand. 1988. Bacterial biodegradation of petroleum hydrocarbons in groundwater: In-situ augmented bioreclamation with enrichment isolates in California. *Water Sci. Technol.* 20:501-503.
- Walton, B. T., and T. A. Anderson. 1988. Structural properties of organic chemicals as predictors of biodegradation and microbial toxicity in soils. *Chemosphere* 17:1501-1507.
- Ward, T. E. 1985. Characterizing the aerobic and anaerobic microbial activities in surface and subsurface soils. *Environ. Toxicol. Chem.* 4:727-737.

- Ward, T. E. 1986. Aerobic and anaerobic biodegradation of nitrilotriacetate in subsurface soils. *Ecotoxicol. Environ. Safety* 11:112-125.
- Werner, P. 1989. Factors limiting the biodegradation of organic compounds in the subsurface during remediation measures. Paper presented at the International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, California.
- Wiggins, B. A., and M. Alexander. 1988. Role of chemical concentration and second carbon sources in acclimation of microbial communities for biodegradation. *Appl. Environ. Microbiol.* 54: 2803-2807.
- Wilson, B. H., B. Bledsoe, and D. Kampbell. 1987. Biological processes occurring at an aviation gasoline spill site. pp. 125-137. IN I.R.C. Avertt and D. M. McKnight (eds.), *Chemical Quality of Water and the Hydrologic Cycle*. Lewis Publishers, Inc., Chelsea, Massachusetts.
- Wilson, B. H., J. T. Wilson, and T. Imbrigiotta. 1989. Effects of codisposal on the biological fate of chlorinated solvents and alkylbenzenes in methanogenic aquifer materials. Paper presented at the International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, California.

- Wilson, J. L., and S. H. Conrad. 1986. Is physical displacement of residual hydrocarbons a realistic possibility in aquifer restoration? IN Proceedings of the National Water Well Association/American Petroleum Institute Conference on Petroleum Hydrocarbons and Organic Chemicals in Groundwater. National Water Well Association, Worthington, Ohio.
- Wilson, J. L. 1989. The behavior of essentially immiscible fluids in contamination problems. Paper presented at the International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, California.
- Wilson, J. T., and J. F. McNabb. 1983. Biological transformation of organic pollutants in groundwater. EOS 64:505-507.
- Wilson, J. T. and B. H. Wilson. 1985. Biotransformation of trichloroethylene in soil. Appl. Environ. Microbiol. 49:242.
- Wilson, J. T. 1989. Biological processes. Paper presented at the International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, California.
- Wilson, S. B., and R. A. Brown. 1989. In-situ bioreclamation: A cost-effective technology to remediate subsurface organic contamination. Ground Water Monit. Rev., Winter:173-179.
- Zaidl, B. R., Y. Murakami, and M. Alexander. 1988. Factors limiting success of inoculation to enhance biodegradation of low concentrations of organic chemicals. Environ. Sci. Technol. 22:1419-1425.

SELECTED BIBLIOGRAPHY

- Borden, R. C., and P. B. Bedient. 1987. In-situ measurements of adsorption and biotransformation at a hazardous waste site. *Water Resour. Bull.* 23:629-636.
- Celia, M. A., J. S. Kindred, and I. Herrera. 1989. Contaminant transport and biodegradation: 1. A numerical model for reactive transport in a porous media. *Water Resour. Res.* 25:1141-1148.
- Cho, J. H. and P. R. Jaffe. 1989. An examination of modeling strategies gas-aqueous phase partitioning of trichloroethylene in unsaturated soils during infiltration. Paper presented at the International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, California.
- Giesy, J. P., S. M. Bartell, P. F. Landrum, G. J. Laversee, and J. W. Bowling. 1983. Fates and biological effects of polycyclic aromatic hydrocarbons in aquatic systems. EPA-600/3-83-053. U.S. Environmental Protection Agency.
- Irvine, R. L., S. A. Sojka, and J. F. Colaroustolo. 1984. Enhanced biological treatment of leachates from industrial landfills. *Hazard. Waste* 1:123-135.
- Knezovick, J. P., J. M. Hirabayashi, D. J. Bishop, and F. L. Harrison. 1988. The influence of different soils types on the fate of phenol and its biodegradation products. *Chemosphere* 17:2199-2205.

- Lynch, N. A., G. G. Wilbur, G. F. Parkin, and J. L. Schnoor. 1989. Biotransformation of pesticides and toxic chemicals in the subsurface environment under aerobic, anoxic and methanogenic conditions. Paper presented at the International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, California.
- Odenerantz, J. E., A. J. Valocchi, W. Bae, and B. E. Rittmann. 1989. Biodegradation of halogenated solvents by biologically active zones induced by nitrate injection into porous-medium flow: II. Computer modeling. Paper presented at the International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, California.
- Olson, B. H., R. A. Goldstein, and D. B. Porcella. 1989. In-situ genetic manipulation to enhance biodegradation. Paper presented at the International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, California.
- Peterson, T. C. 1989. Bacterial retention in soils. *J. Environ. Health* 51:196-200.
- Phipps, D. W. and H. F. Ridgway. 1989. Aerobic gasoline biodegradation studies using computer-controlled, non-dispersive infrared CO₂ analysis. Paper presented at the International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, California.

- Powell, R. M., R. W. Callaway, J. T. Michalowski, S. A. Vandegrift, M. V. White, D. H. Kampbell, B. E. Bledsoe, and J. T. Wilson. 1988. Comparison of methods to determine oxygen demand for bioremediation of a fuel contaminated aquifer. *Int. J. Environ. Anal. Chem.* 34:253-263.
- Reinhard, M., N. L. Goodman, and J. F. Barker. 1984. Occurrence and distribution of organic chemicals in two landfill leachate plumes. *Environ. Sci. Technol.* 18:953-961.
- Reinhard, M., F. Haag, and P. L. McCarty. 1989. Selective degradation of toluene and p-xylene in an aerobic microcosm. Paper presented at the International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, California.
- Rifai, H. S. and P. B. Bedient. 1989. Comparison of two conceptual models for simulating biodegradation of organic contaminants in groundwater. Paper presented at the International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, California.
- Ryan, J. A., R. M. Bell, J. M. Davidson, and G. A. O'Connor. 1988. Plant uptake on non-ionic organic chemicals from soils. *Chemosphere* 17:2299-2323.
- Schwarzenbach, R. 1989. Abiotic processes. Paper presented at the International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, California.

- Tabak, H. H., S. A. Quave, C. I. Mashni, and E. F. Barth. 1981. Biodegradability studies with organic priority compounds. *J. Water Pollut. Control Fed.* 53:1503-1518.
- Tam, E., and W. J. Maler. 1989. Biodegradation of naphthalene and distribution of biomass in continuous flow unsaturated sand columns. Paper presented at the International Symposium on Processes Governing the Movement and Fate of Contaminants in the Subsurface Environment, Stanford University, Stanford, California.
- Vaishnav, D. D., and L. Babeu. 1987. Comparison of occurrence and rates of chemical biodegradation in natural waters. *Bull. Environ. Contam. Toxicol.* 39:237-244.
- Walton, B. T., T. A. Anderson, M. S. Hendricks, S. S. Talmage. 1989. Physiochemical properties and predictors of organic chemical effects on soil microbial respiration. *Environ. Toxicol. Chem.* 8:53-63.
- Walton, B. T., M. S. Hendricks, T. A. Anderson, and S. S. Talmage. 1989. Treatability of hazardous chemicals in soils: Volatile and semivolatile organics. ORNL-6451.
- Webster, P. 1986. Enclosed thermal soil aeration for removal of volatile organic contamination. *J. Air Pollut. Assoc.* 36:1156-1168.
- Wilson, J. T., and D. H. Kampbell. 1989. Challenges to the practical application of biotechnology for the biodegradation of chemicals in groundwater. pp. 74-76. IN Proc., 194th American Chemical Society National Meeting, Dallas, Texas.

INTERNAL DISTRIBUTION

- | | |
|-----------------------|---------------------------------|
| 1. T. L. Ashwood | 16. J. E. Peterson |
| 2. L. D. Bates | 17. D. A. Pickering |
| 3. B. A. Berven | 18. D. E. Reichle |
| 4. J. H. Cushman | 19. D. S. Shriner |
| 5. K. S. Dickerson | 20. R. L. Siegrist |
| 6. M. P. Farrell | 21. D. R. Smuin |
| 7. D. W. Foster | 22. S. H. Stow |
| 8. S. B. Garland | 23. R. I. Van Hook |
| 9. C. W. Gehrs | 24. Central Research Library |
| 10. S. C. Hall | 25-39. ESD Library |
| 11. S. G. Hildebrand | 40-41. Laboratory Records Dept. |
| 12. A. D. Laase | 42. Laboratory Records, ORNL-RC |
| 13. C. A. Little | 43. ORNL Patent Office |
| 14. A. P. Malinauskas | 44. ORNL Y-12 Technical Library |
| 15. C. A. Muhr | |

EXTERNAL DISTRIBUTION

45. D. E. Brown, Allied Signal, Inc., Dept. 922, MC SC8, 2000 E. 95th Street, Kansas City, MO 64131
46. E. Brown, Allied Signal, Inc., 2000 E. 95th Street, Kansas City, MO 64131
47. Q. Fernando, University of Arizona, University Analytical Center, Department of Chemistry, Tucson, AZ 85721
48. J. F. Franklin, Bloedel Professor of Ecosystem Analysis, College of Forest Resources, University of Washington, Anderson Hall (AR-10), Seattle, WA 98195
49. P. Hoopes, DOE KC Area Office, 2000 E., 95th Street/P.O. Box 202, Kansas City, MO 64131
50. G. M. Hornberger, Professor, Department of Environmental Sciences, University of Virginia, Charlottesville, VA 22903
51. G. Y. Jordy, Director, Office of Program Analysis, Office of Energy Research, ER-30, G-226, U.S. Department of Energy, Washington, DC 20545
52. P. M. Kearl, ORNL-Grand Junction, P.O. Box 2567, Grand Junction, CO 81502
53. P. Keary, DOE KC Area Office, 20000 E. 95th Street, Kansas City, MO 64131
- 54-79. N. E. Korte, ORNL-Grand Junction, P.O. Box 2567, Grand Junction, CO 81502
80. G. E. Likens, Director, The New York Botanical Garden, Institute of Ecosystem Studies, The Mary Flagler Gary Arboretum, Box AB, Millbrook, NY 12545
81. H. M. McCammon, Director, Ecological Research Division, Office of Health and Environmental Research, Office of Energy Research, ER-75, U.S. Department of Energy, Washington, DC 20545

82. D. Neff, EG&G, P.O. Box 3000, Miamisburg, OH 45343
83. S. Ritchey, USEPA Region VII, 726 Minnesota Avenue, Kansas City, KS 66101
84. M. Stites, Allied Signal, Inc., Dept. 922 MC SC8, 2000 E. 95th Street, Kansas City, MO 64131
85. D. L. Stoltz, Allied Signal, Inc., 2000 E. 95th Street, Kansas City, MO 64131
86. B. Stuart, Wasteman Program MDNR, P.O. Box 176, Jefferson City, MO 65102
87. S. Wagner, Los Alamos National Lab, P.O. Box 1663, MS-K485, Los Alamos, NM 87544
88. S. White, Allied Signal, Inc., Dept. 922 MC SC8, 2000 E. 95th Street, Kansas City, MO 64131
89. F. J. Wobber, Ecological Research Division, Office of Health and Environmental Research, Office of Energy Research, ER-75, U.S. Department of Energy, Washington, DC 20545
90. Office of Assistant Manager for Energy Research and Development, Oak Ridge Operations, P.O. Box 2001, U.S. Department of Energy, Oak Ridge, TN 37831-8600
- 91-100. Office of Scientific and Technical Information, P.O. Box 62, Oak Ridge, TN 37831